

**UCLA**

**UCLA Electronic Theses and Dissertations**

**Title**

Macroparasite Study of Cypriniform fishes in the Santa Clara Drainage

**Permalink**

<https://escholarship.org/uc/item/3kp0q16j>

**Author**

Murray, Max DeLonais

**Publication Date**

2019

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Macroparasite Study of Cypriniform fishes  
in the Santa Clara Drainage

A thesis submitted in partial satisfaction of the  
requirements for the degree Master of Science  
in Biology

by

Max DeLonais Murray

2019

© Copyrite by

Max DeLonais Murray

2019

## ABSTRACT OF THE THESIS

### Macroparasite Study of Cypriniform fishes in the Santa Clara Drainage

by

Max DeLonais Murray

Master of Science in Biology

University of California, Los Angeles, 2019

Professor Donald G. Buth, Chair

Several species of fishes have been introduced into the Santa Clara River system in southern California, including *Catostomus santaanae* (Santa Ana sucker), *Catostomus fumeiventris* (Owens sucker), *Gila orcutti* (arroyo chub), and *Pimephales promelas* (fathead minnow). These species are known to inhabit similar ecological niches but little is known about their associated parasite fauna. Two *C. fumeiventris*, 35 *C. santaanae*, 63 hybrid catostomids, 214 *G. orcutti*, and 18 *P. promelas* were collected and necropsied in the summers of 2017 and 2018. Nine macroparasite taxa were harvested including seven native, and two nonnative parasites *Schyzocotyle acheilognathi* (Asian fish tapeworm) and *Lernaea cyprinacea* (anchor worm). Prevalence and intensity of parasites were not related to the genetic history of these catostomids. This is the first host-association record for *G. orcutti* with *Gyrodactylus* sp., *S. acheilognathi*,

diplostomid metacercariae, *Rhabdochona* sp, *Contracaecum* sp., and larval acuariid cysts and for *P. promelas* with larval acuariid cysts.

The thesis of Max DeLonais Murray is approved.

Jamie Lloyd-Smith

Pamela Yeh

Donald G. Buth, Chair

University of California, Los Angeles

2019

## TABLE OF CONTENTS

<b>CHAPTER 1 - MACROPARASITE FAUNA OF <i>CATOSTOMUS FUMEIVENTRIS</i>, <i>C. SANTAANAE</i>, AND THEIR HYBRIDS (ACTINOPTERYGII: CATOSTOMIDAE) IN THE SANTA CLARA RIVER, VENTURA COUNTY, CALIFORNIA.....</b>	<b>1</b>
<b>INTRODUCTION.....</b>	<b>1</b>
<b>MATERIALS AND METHODS.....</b>	<b>3</b>
<b>RESULTS .....</b>	<b>6</b>
<b>DISCUSSION .....</b>	<b>7</b>
Table 1. Host size (in mm SL), genetic makeup (% of genes of <i>C. fumeiventris</i> ), and collection locality. ....	15
Table 2. Prevalence % (P), mean intensity (MI), median intensity (Mdn I) and minimum and maximum counts (Min - Max) of macroparasites associated with parental <i>C. santaanae</i> , F1 hybrids, F2 hybrids, and parental <i>C. fumeiventris</i> . ....	16
Figure 1. Maps of study locality. ....	17
Figure 2. Distribution of five marker loci in 100 catostomid fishes collected from the Santa Clara Watershed. Number of individuals expressing a percentage of “genes of <i>C. fumeiventris</i> ” are shown: 0% = <i>C. santaanae</i> and 100% = <i>C. fumeiventris</i> . ....	18
Figure 3. Average heterozygous loci of infected and not infected hybrid catostomids from the Santa Clara Watershed. ....	19
Figure 4. Prevalence of encysted acuariid nematodes, <i>Ornithodiplostomum</i> sp., <i>L. cyprinacea</i> , <i>Rhabdochona</i> sp., <i>S. acheilognathi</i> from catostomid hosts in the Santa Clara Watershed. ....	20
Figure 5. Median intensity of encysted acuariid nematodes, <i>Ornithodiplostomum</i> sp., <i>L. cyprinacea</i> , <i>Rhabdochona</i> sp., <i>S. acheilognathi</i> from catostomid hosts in the Santa Clara Watershed. ....	21
<b>LITERATURE CITED.....</b>	<b>22</b>
 <b>CHAPTER 2 - MACROPARASITES OF <i>GILA ORCUTTI</i> AND <i>PIMEPHALES PROMELAS</i> (ACTINOPTERYGII: CYPRINIDAE) IN THE SANTA CLARA RIVER, VENTURA COUNTY, CALIFORNIA.....</b>	<b>31</b>
<b>INTRODUCTION.....</b>	<b>31</b>
<b>MATERIALS AND METHODS.....</b>	<b>32</b>
<b>RESULTS .....</b>	<b>34</b>
<b>DISCUSSION .....</b>	<b>35</b>

Table 1. Prevalence % (P ), mean intensity (MI), median intensity (Med I), minimum - maximum counts (Min - Max), of parasites collected from cypriniform fishes in the Santa Clara River. ....	39
<b>LITERATURE CITED.....</b>	<b>41</b>



# **Chapter 1 - Macroparasite Fauna of *Catostomus fumeiventris*, *C. santaanae*, and their Hybrids (Actinopterygii: Catostomidae) in the Santa Clara River, Ventura County, California.**

## **INTRODUCTION**

The Santa Clara River is one of the largest and least developed coastal drainages in southern California (Beller et al., 2011). However, in 1928 the St. Francis Dam failure released water from the California Aqueduct directly into the Santa Clara River, which altered the stream morphology and ecology of the Santa Clara watershed (Begnudelli and Sanders, 2007; Beller et al., 2011). This flooding event released Owens River water and the organisms that it carried into the Santa Clara River. Introductions of nonnative species can have lasting negative effects both directly and indirectly on the endemic species (Moyle, 1976; Marchetti et al., 2004; Kock et al., 2010; Howard et al., 2015; Grantham et al., 2017).

Thirty species of fishes have been reported from the Santa Clara River, including six native species and twenty-four introduced species (Moyle, 2002). Two distantly related catostomids, *Catostomus fumeiventris* and *C. santaanae*, are now known to be established in the lower portions of the Santa Clara and its tributaries (Bell, 1978; Richmond et al., 2018). Both *C. fumeiventris* and *C. santaanae* are considered to be introduced to the Santa Clara River with the breach of the California Aqueduct likely leading to the introduction of *C. fumeiventris* (Bell, 1978; Moyle, 2002; Richmond et al., 2018). However, the status and origin of the *C. santaanae* population has been debated (Bell, 1978; Richmond et al., 2018). The population of *C. santaanae* in the Santa Clara River has been shown to hybridize with the introduced *C. fumeiventris* in the lower portions of the Santa Clara River down-stream of Filmore, CA (Hubbs,

1955; Smith, 1966; Bell, 1978; Buth and Crabtree, 1982; Buth et al., 2008; Chabot et al., 2009; Richmond et al., 2018). Hybridization is common in cypriniform fishes of North America and has been well documented in catostomids across the country (Hubbs, 1955; Smith, 1992; Bangs et al., 2018). The hybridization of *C. fumeiventris* and *C. santaanae* in the Santa Clara River is the only documented instance of hybridization of these two species.

Studies on the ecology of *C. santaanae* in the Los Angeles, San Gabriel, and Santa Ana watersheds have focused on the effect that urbanization has had on the threatened populations within these rivers (Greenfield et al., 1970; Saiki et al., 2007; Jenkins et al., 2009; Thompson et al., 2010). Despite the in-depth knowledge on the effects of habitat alteration on abundances of *C. santaanae*, there has been relatively few studies focusing on parasites and infectious disease that affect *C. santaanae*. The same can be said about *C. fumeiventris* although there has been much less research on its distribution and ecology within the Owens River (Miller, 1973; Moyle, 2002).

Recently several studies have focused on the distributions and host associations of three nonnative pathogenic parasites: *Ichthyophthirius multifiliis* (white spot disease), *Schyzocotyle acheilognathi* (Asian fish tapeworm), and *Lernaea cyprinacea* (anchor worm) in coastal drainages of southern California (Warburton et al., 2001; Kuperman et al., 2002). Documenting the distributions of *I. multifiliis*, *S. acheilognathi*, and *L. cyprinacea* is vitally important for resource managers as all three parasites have been shown to have negative effects on infected populations of fish and amphibians (Kabata, 1979; Hoffman, 1999; Hansen et al., 2006; Kupferberg et al., 2009; Archdeacon et al., 2010; Tufail et al., 2017; Kuchta et al., 2018). While these studies have focused on the distributions and host associations of *I. multifiliis*, *S. acheilognathi*, and *L. cyprinacea* on *C. santaanae* in southern California, little is known about

the other parasitic fauna that might infect *C. santaanae* and there are no known records of parasite associations with *C. fumeiventris* (Hoffman, 1999; Warburton et al., 2001).

This study provides a comprehensive survey of the macroparasites that are associated with the *C. fumeiventris* and *C. santaanae* in the Santa Clara River. Additionally, the macroparasite communities of pure *C. fumeiventris*, pure *C. santaanae*, and their hybrids will be compared in order to address the specificity of parasitic fauna associated with these catostomids found in the Santa Clara River.

## **MATERIALS AND METHODS**

### **Study area**

The study area included two sampling sites, the mainstem of the Santa Clara River and Sespe Creek, in Ventura County California (Figure 1). The sampling locality in the mainstem of the river was characterized by a dense riparian buffer that consisted of large willows and Spanish cane that created a canopy over most of the river. The banks were dominated by emergent vegetation and deep undercuts in slower portions of the river. Sespe Creek was characterized by an open canopy with a narrow corridor of willows bordering the creek. The banks were steep with sparse emergent vegetation. The sampling efforts took place from June to September in 2017 and from May to September in 2018. The mainstem of the Santa Clara River was only able to be sampled during August and September of 2018. Water temperature was recorded in Sespe Creek with the Onset® HOBO® Water Temp Pro v2 temperature logger. The temperature logger was set to record temperatures every thirty minutes during the project period.

### **Sampling**

*Fishes:* Fishes were caught using a 5 m two-person seine net with a nylon delta weave 5 mm mesh size. In accordance with the California Department of Fish and Wildlife, a specialized seine protocol was developed in order to minimize disturbance to potential southern California steelhead habitat. Sampling efforts were limited to fifty seine hauls in each 50-meter reach. Additionally, sampling efforts were restricted to one effort per month during the sampling period. Lethal take was highly regulated by the California Department of Fish and Wildlife with the maximum number of *C. fumeiventris* and *C. santaanae* being limited to 35 individuals per sampling effort.

Fishes were tentatively identified, counted, and placed in 19 L buckets with aerators before processing. Identification was initially based on morphological features in the field, however allozyme gel electrophoresis was required because of the uncertain genetic history of these fishes. Standard length was measured, then the fishes were necropsied using dissecting microscopes. Vouchers were deposited in the Los Angeles County Natural History Museum Ichthyology Collection (LACM 60398-1). The protocol for genetic identification of hybrids followed methods described in Buth et al. (2008). Brain and muscle tissue were extracted and used for starch gel electrophoresis. Five marker loci were used for identification: acid phosphatase (Acp-A), creatine kinase (Ck-A), two esterases (Est - 1, and Est - 2), and glycerol-3-phosphate dehydrogenase (G3pdh-A). Counts of heterozygous loci were used to estimate the percentage of genes shared by the hybrid catostomids. Parental catostomids were characterized by homozygous expression at all marker loci, F<sub>1</sub> hybrids were characterized by heterozygous expression at all marker loci, and F<sub>2</sub> hybrids were characterized by a mix of homozygous and heterozygous expression of the marker loci.

*Parasites*: External surfaces, and internal organs including the stomach, intestine, and gallbladder were examined for parasites. Parasites were fixed and preserved using methods described in Dailey (1996) and Justine et al. (2012). Platyhelminths, acanthocephalans, and nematodes were preserved for both morphological and molecular analysis. Copepods were collected and placed directly into either 70% or 95% ethanol. Parasites were identified with Commonwealth Institute of Helminthology (1974), Hoffman (1999), and Kabata (1979).

## Data Analysis

Parasite prevalence, mean intensity, and median intensity (Bush et al., 1997) were calculated for each host. Parasite distributions were aggregated, thus rendering mean intensity an inaccurate descriptor of parasite intensity in the host population (Reiczigel et al., 2019). A generalized linear model was used to analyze the relationship between host standard length and parasite intensity. Permutation tests were used to analyze differences in prevalence, and median intensity between hosts (Crowley, 1992; Gotelli, 2000; Thomas and Poulin, 1997). Observed values were randomly shuffled without replacement keeping sample size constant for 10,000 iterations. Difference values of each iteration were compiled to create a distribution of potential differences. Observed differences in prevalence and median intensity were then compared to their respective probability distributions from the permutation to obtain a probability value. Bootstrapped ninety-five percent confidence intervals were calculated using 10,000 iterations for prevalence and median intensity values. Median intensity confidence intervals were displayed when parasite intensity was variable. Data analysis was performed with the “tidyverse”, “hms”, “lubridate” and “infer” packages in R (Grolemund and Wickham, 2011; Wickham, 2017; Bray et al., 2018; Müller, 2018; R Core Team, 2018).

## RESULTS

During this course of this study 100 catostomids were collected from the lower Santa Clara River watershed. Both *C. fumeiventris* and *C. santaanae* were collected as well as F<sub>1</sub> and F<sub>2</sub> hybrids. The F<sub>2</sub> hybrids and parental *C. santaanae* were present at both localities, however F<sub>1</sub> hybrids and parental *C. fumeiventris* were only found in the mainstem of the Santa Clara River (Table 1, Figure 2). Fishes collected in the mainstem of the river were larger than fishes from Sespe Creek ( $P < 0.0001$ ). However, parasite intensity was not consistently related with fish size ( $X^2 = 1.825$ ,  $df = 1$ ,  $P = 0.177$ ). The prevalence of infection and median intensity did not differ with genetic status of the hosts ( $P = 1.00$ ) (Figure 3).

A total of 226 individual parasites were collected with eight species represented during this study (Table 2). Four of the eight species of parasites collected were found as adults. One species, *L. cyprinacea*, was collected in two life cycle stages: adult females and copepodids. A single specimen of *Isoglaridacris* sp. was collected from an F<sub>2</sub> hybrid sucker from Sespe Creek, while a single cystacanth was collected from an F<sub>2</sub> hybrid sucker in the mainstem of the river (Table 2). The most common parasite collected during this study was the nonnative copepod *L. cyprinacea* with a total of 112 individuals; 37 adults and 74 copepodids (Table 2). The prevalence of adult *L. cyprinacea* ranged from 5.71% in *C. santaanae* to 100% in *C. fumeiventris* (Table 1, Figure 4). The prevalence of *L. cyprinacea* copepodids was also similar between hosts (Table 2). The next most common parasite was *S. acheilognathi*, which also had a similar prevalence among all hosts ( $P = 0.554$ ) (Table 2, Figure 4). Diplostomid metacercariae, identified as a species of *Ornithodiplostomum*, were collected consistently from parental *C.*

*santaanae* and F<sub>2</sub> hybrids in Sespe Creek with a prevalence of 8.6% and 9.83%, respectively ( $P = 1.00$ ) (Table 2, Figure 4).

Two species of nematodes were collected during this study. Larval acuariid cysts were collected from the viscera surrounding the intestine, whereas the *Rhabdochona* sp. were collected from the lumen of the intestines. The prevalence of *Rhabdochona* sp. was consistent between hosts and ranged from 5.71% to 8.20% ( $P = 1.00$ ) (Figure 4). The prevalence of the acuariid cysts was also consistent between hosts and ranged from 11.42% in *C. santanae* to 16.39% in the F<sub>2</sub> hybrids (Figure 4). The median intensity of the acuariid nematodes was consistent between *C. santaanae* and F<sub>2</sub> hybrids ( $P = 0.989$ ) (Figure 5).

## DISCUSSION

The parasite diversity in the Santa Clara River is higher than expected with five of the eight species of parasites collected during this study being new records for host-parasite associations. The two nonnative parasites, *Schyzocotyle acheilognathi* and *Lernaea cyprinacea*, have both been reported to be found in association with other fishes in the Santa Clara River (Warburton et al., 2001; Kuperman et al., 2002). However, only *L. cyprinacea* has been known to infect *Catostomus santaanae* from the Santa Ana River (Warburton et al., 2001; Kuperman et al., 2002). Previous to this study, there are no published records of any parasite associations with *C. fumeiventris* and while this host has been introduced to the Santa Clara River there is no reason to believe that both *S. acheilognathi* and *L. cyprinacea* are not present in the Owens River Watershed as there are several species of fishes that have been introduced to the Owens River that are well documented as final hosts of both parasites (Moyel, 2002; Kuchta et al., 2018).

Nonnative parasites were the most common parasites in this study, which is in agreement with other parasite surveys of native fishes in the western United States (Amin, 1969; Wier et al., 1983; Robinson et al., 1998; Choudhury et al., 2004). The results of this study show that *S. acheilognathi* did not differentially infect parental *C. santaanae* and hybrid catostomids in the Santa Clara River. The global distribution, ability to infect a wide range of both intermediate and final hosts, as well as its apparent ability to develop in a wide range of environmental conditions has categorized *S. acheilognathi* as being considered the most widespread and successful parasite of freshwater fishes (Kuchta et al., 2018). It was not previously known to infect *C. santaanae*, but was reported from other fishes in the Santa Clara River, Los Angeles River, San Gabriel River, and Santa Ana River (Kuperman et al., 2002). These findings are troubling but not surprising due to the fact that *S. acheilognathi* is known to infect fishes across thirty-eight families on every continent other than Antarctica (Kuchta et al., 2018). While most fishes that had a low numbers *S. acheilognathi* and seemed to be in good condition upon collection, fishes that had more than one cestode had damaged or distended stomachs, which may be an early indication of secondary infection or lesions in the stomach wall.

Adult and copepodid *L. cyprinacea* were collected during this study and were found in association with all hosts, with the exception of *C. fumeiventris* having no copepodids associated with the two host specimens collected. However, this is likely due to the low abundance and sample size of *C. fumeiventris* in the lower Santa Clara River. Copepodid stages are highly mobile and still possess the ability to move from one host to another whereas adult *L. cyprinacea* are mesoparasitic, and can be found imbedded in the host. Adult *L. cyprinacea* have been shown to cause mortality in fish and amphibian hosts caused by a reduced swimming performance, damage to host internal organs, and secondary infection (Barber et al., 2000; Tufail et al., 2017;



Welicky et al., 2017; Waicheim et al., 2019). Copepodid stages of *L. cyprinacea* are difficult to quantify, and the effects of the copepodids on fish hosts is not well documented. Some studies have correlated fish die-offs and damage to fish gill filaments with high intensities of copepodids of *L. cyprinacea*, however it is not well understood if the damage to the host is caused by copepodids feeding on host tissue or the process of the female copepodids imbedding and metamorphizing into adults (Shields and Tidd, 1974; Hossain et al., 2018). Upon collection, fishes that had adult *L. cyprinacea* imbedded into the base of the pectoral or dorsal fins seemed to show altered swimming performance, with some of the most highly infected fishes not being able to control buoyancy or keep their position in faster moving water in Sespe Creek. Attachment point of adult *L. cyprinacea* has been studied in the past, however, more research needs to focus on the hydrodynamic effects that this mesoparasitic copepod has on fishes swimming performance (Shields and Tidd, 1974).

River systems in southern California exhibit ephemeral surface water with sections of the rivers going completely dry leaving isolated pools and relatively small reaches of flowing water. Fishes native to the southwestern United States have adapted to this unique flow regime, which may provide parasites with direct life cycles, like *L. cyprinacea*, with ideal conditions to spread as host populations during these low flow cycles can be concentrated in small areas of the streambed. Studies of intermittent streams in northern California and Brazil show that *L. cyprinacea* intensity significantly increases during periods of seasonal warm water and low surface water flows (Medeiros and Maltchik, 1999; Kupferberg et al., 2009). With climate change and an increased demand for freshwater, the presence of *L. cyprinacea* may become an increasing issue for the conservation of native fishes in California as well as the southwestern United States.

Native parasites were relatively uncommon among the catostomids in the Santa Clara River. The origin of the populations of *C. santaanae* in the Santa Clara River could have affected the number of native parasites that were observed in this study, as it is likely that not all parasites would have become established upon the introduction of their host. All species of native parasites, with the exception of the single specimen of *Isoglaridacris* sp., were not fully reproductive adults. The digeneans collected from the catostomids in the Santa Clara were encysted metacercariae from the intestinal mesentery, near the gas bladder, and the liver. Brain tissue was not examined for metacercarial cysts due to the allozyme protocol requiring this tissue. This may have confounded the results for prevalence and intensity in part due to this digenean's ability to migrate to the brain. The metacercarial cysts are difficult to identify to species level with morphological data at this life cycle stage. However, during dissection some metacercariae excysted and were subsequently identified as a species in the genus *Ornithodiplostomum*. The metacercarial stage of the digenean life cycle is typically referred to as a resting stage with little interaction between the host and parasite (Matisz and Goater, 2010). However, studies have shown that some species within this genus, particularly the species *Ornithodiplostomum ptychocheilus*, are known to migrate to the brain of the fishes it infects presumably to alter host behavior and increase the probability of transmission its final host: a piscivorous bird (Hoffman, 1958; Radabaugh, 1980; Matisz and Goater, 2010).

Two species of rare parasites were collected during this study, a cystacanth stage of an acanthocephalan and an adult caryophyllid cestode from the genus *Isoglaridacris*. Only one specimen of each species was collected from F<sub>2</sub> hybrids. The acanthocephalan was not able to be identified as it was completely encysted. This is the first record of an acanthocephalan being collected from catostomids in southern California. Acanthocephalans can have a complex life

cycle, that usually starts with an arthropod first intermediate host and in this case, it is likely that the fish host is the second intermediate host and the final host is either a piscivorous fish or bird (Dailey, 1996). Cystacanths have been reported to modify the behavior of their intermediate hosts, however invertebrate intermediate hosts have been the focus of the majority of these studies (Bethel and Holmes, 1973).

The caryophyllid cestode is the first specimen of this family to be reported from southern California fishes. This group of cestodes is thought to have an oligochaete intermediate host and a fish final host, however this group has not been well studied in the southwestern United States (Mackiewicz, 1972; Hoffman, 1999). While the specimen collected in this study has been identified as a species in the genus *Isoglaridacris*, identification to the species level was not possible with only one specimen collected. This genus of caryophyllid cestodes can be found associated with several catostomid species across the United States (Mackiewicz, 1972; Hoffman, 1999).

The nematodes collected in this study were the first records of nematodes associated with the *C. santaanae* and the hybrids found in the Santa Clara River. They represented one of the most common groups of parasites among the catostomid hosts from this study. Nematodes in the family Acuariidae, which were found encysted in the intestinal viscera, are common parasites of aquatic birds. The life cycle of acuariids typically involves an arthropod intermediate host that is either consumed directly by the final host or is consumed by a fish or amphibian paratenic host then is finally consumed by a piscivorous bird (Anderson, 1988). The second species of nematode identified to the genus *Rhabdochona* was collected from the lumen of the intestine in both F<sub>2</sub> hybrid and parental *C. santaanae*. This genus of nematode has been described to have a life cycle that is comprised of an aquatic insect intermediate host with the final host being a fish

(Anderson, 1988; Moravec, 2010). No gravid adult female *Rhabdochona* sp. were collected from catostomid hosts during this study. All specimens were either infective larval stages or young adult males. This genus is typically found in association with cyprinids in North America (Hoffman, 1999) and the specimens infecting the catostomids in the Santa Clara River could be representative of a situation where the intermediate host containing the infective larval stage of *Rhabdochona* sp. is being consumed and results in a dead-end for this parasite's life cycle. The morphological features of the specimens of *Rhabdochona* sp. are similar to other species that are closely associated with cyprinids in North America (Moravec and Huffman, 1988; Moravec, 2010). However, more research focused on the morphological features as well as the genetic identity of this nematode is required to identify it to the species level.

The prevalence and intensity of parasites from this study were not related to the genetic history of the catostomids in the Santa Clara River. However, the sample size of the *C. fumeiventris* and F<sub>1</sub> groups do not allow for accurate detection of differences in the parasite infections. The data suggest that the life history of the parasites and possibly the relative abundance of infected intermediate hosts is more likely to influence the infection parameters of the parasites collected in this study. For example, the two nonnative species, *S. acheilognathi* and *L. cyprinacea*, are generalists and thus it is not likely that hybrid catostomids would be differentially infected by either of these invasive parasites (Hoffman, 1999; Kuchta et al., 2018). The native parasites from this study are utilizing the catostomids as either intermediate hosts, final hosts, or potentially dead-end hosts. While more research is needed on the specific life cycles of these parasites the general life cycles of the digenean, acanthocephalan, and acuariid nematode suggest that the catostomids are being eaten by piscivorous birds that may also suggest that these parasites are likely to be found in adjacent watersheds in southern California due to the

mobile nature of the final hosts. The specimen of *Isoglaridacris* was the only fully mature native parasite collected during this study, which suggests that this cestode is able to at least establish in the catostomid hosts. Studies focusing on the life cycle of other species of *Rhabdochona* have suggested that this group is transmitted trophically to its fish final host through an insect in the order Plecoptera or Ephemeroptera (Anderson, 1988). The life cycle stages collected during this study suggest that either the catostomids were all newly infected with larval *Rhabdochona* or that the catostomids represent a dead-end host where the nematodes are not able to develop into reproductive adults (Anderson, 1988). More research is needed to describe the life cycle of this species of *Rhabdochona* as well as a thorough morphological and genetic study to investigate its phylogenetic relationship with its congeners.

This study provides comprehensive base-line data on the macroparasite fauna of *C. fumeiventris*, *C. santaanae*, and their hybrids. While both *C. fumeiventris* and *C. santaanae* are not likely to be native to the Santa Clara watershed, this study highlights nonnative and native parasite associations with the hosts. Nonnative parasites can be a serious threat to management and recovery of special status species and monitoring invasive parasites is imperative especially in the case of freshwater fishes in southern California (Moyle, 1976; Brooks and Hoberg, 2000; Kupferberg et al., 2009; Kock et al., 2010; Tufail et al., 2017; Kuchta et al., 2018). It is unlikely that these parasites can be eradicated. However, with consistent monitoring efforts, management plans can be implemented to reduce the population wide infection rates (Henriksen et al., 2019). Native parasites can be useful bioindicators for trophic interactions, studies of population structure, and biogeographical studies, but have been neglected historically from studies of native fishes in southern California (Hoffman, 1999; Brooks and Hoberg, 2000; Scholz and Choudhury, 2014). Parasites with complex life cycles have been demonstrated as effective

models for monitoring restoration of wetlands in southern California and this method may be used in freshwater systems to assess habitat restoration projects in southern California (Hudson et al., 2006). Additionally, it is invaluable to conduct parasite surveys in systems that have not yet been studied in this respect like locations in the southwestern United States (Scholz and Choudhury, 2014). Continued and expanded parasite surveys in the Santa Clara River as well as other watersheds in California may lead to new discoveries of both nonnative and native parasite interactions with aquatic organisms endemic to this region.

Table 1. Host size (in mm SL), genetic makeup (% of genes of *C. fumeiventris*), and collection locality.

Locality	<i>C. santaanae</i>		F <sub>1</sub> Hybrid		F <sub>2</sub> Hybrid												<i>C. fumeiventris</i>	
	0%		50%		10%		20%		30%		40%		50%		>50%		100%	
	n	SL	n	SL	n	SL	n	SL	n	SL	n	SL	n	SL	n	SL	n	SL
Sespe Creek	32	42-79			11	41- 77	4	43- 62	16	58- 89	5	47- 98	3	45- 84	5	53-76		
Santa Clara River	3	92- 117	2	121-127	1	122	1	40	3	46- 127	4	118- 102	3	55- 132	5	105-166	2	103- 122

Table 2. Prevalence % (P), mean intensity (MI), median intensity (Mdn I) and minimum and maximum counts (Min - Max) of macroparasites associated with parental *C. santaanae*, F1 hybrids, F2 hybrids, and parental *C. fumeiventris*.

Host	<i>C. santaanae</i> (n = 35)				F <sub>1</sub> Hybrid (n = 2)				F <sub>2</sub> Hybrid (n = 61)				<i>C. fumeiventris</i> (n = 2)			
Parasite	P	MI	Mdn I	Min - Max	P	MI	Mdn I	Min - Max	P	MI	Mdn I	Min - Max	P	MI	Mdn I	Min - Max
Cestoda																
<i>S. acheilognathi</i>	17.14	4.33	4.5	0 - 7					11.46	1.86	1	0 - 6				
<i>Isoglaridacris</i> sp.									1.64	1	1	0 - 1				
Digenea																
<i>Ornithodiplostomum</i> sp.	8.57	1.33	1	0 - 2					9.84	2.67	1	0 - 9				
Acanthocephala																
Unidentified cystacanth									1.64	1	1	0 - 1				
Nematoda																
<i>Rhabdochona</i> sp.	5.71	1	1	0 - 1					8.20	4	1	0 - 16				
Acuarid cyst	11.43	1.75	1	0 - 4					16.39	2.40	1	0 - 16				
Copepoda																
<i>L. cyprinacea</i> (adult)	5.71	1	1	0 - 1	100	1.5	1.5	1-2	24.59	1.5	1	0 - 3	100	3	3	0 - 4
<i>L. cyprinacea</i> (copepodid)	14.29	1.4	1	0 - 3	50	7	7	0 - 7	9.84	10	2	0 - 40				



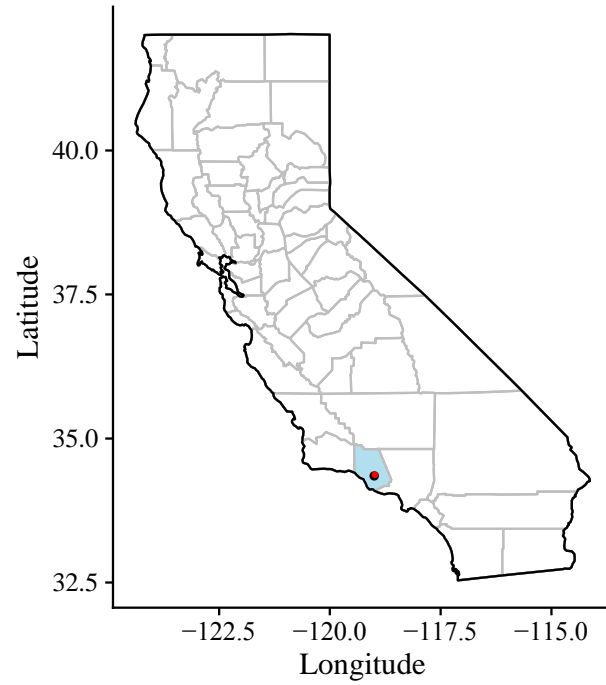
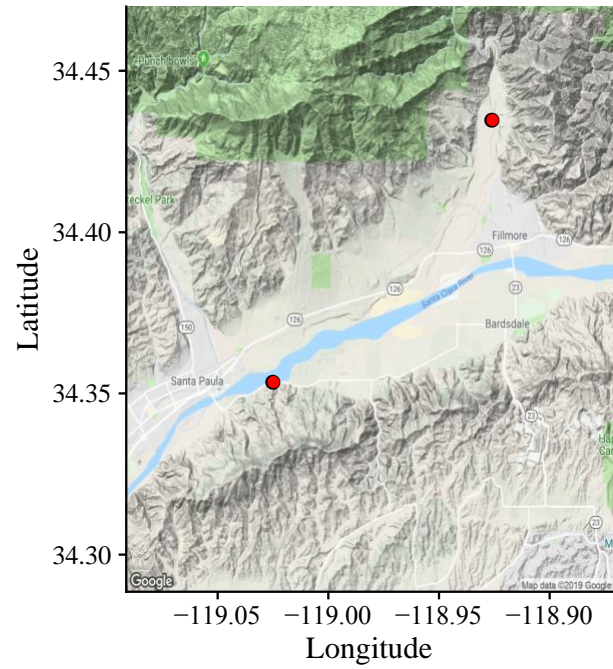
**A****B**

Figure 1. Maps of study locality. A) Ventura County, California shaded blue and Filmore, California as a red dot. B) Collecting localities in Sespe Creek and the mainstem of the Santa Clara River.

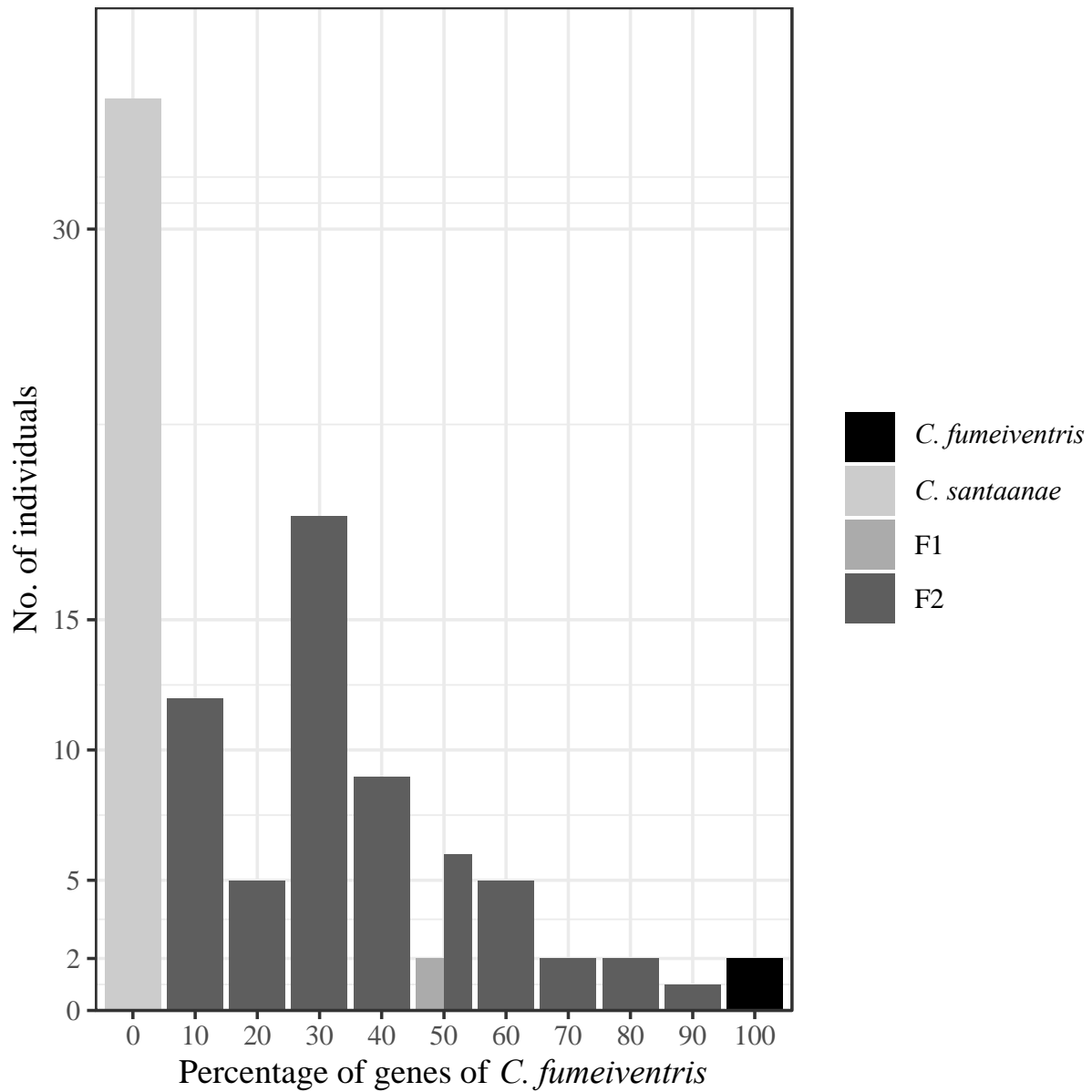


Figure 2. Distribution of five marker loci in 100 catostomid fishes collected from the Santa Clara Watershed. Number of individuals expressing a percentage of “genes of *C. fumeiventris*” are shown: 0% = *C. santaanae* and 100% = *C. fumeiventris*.

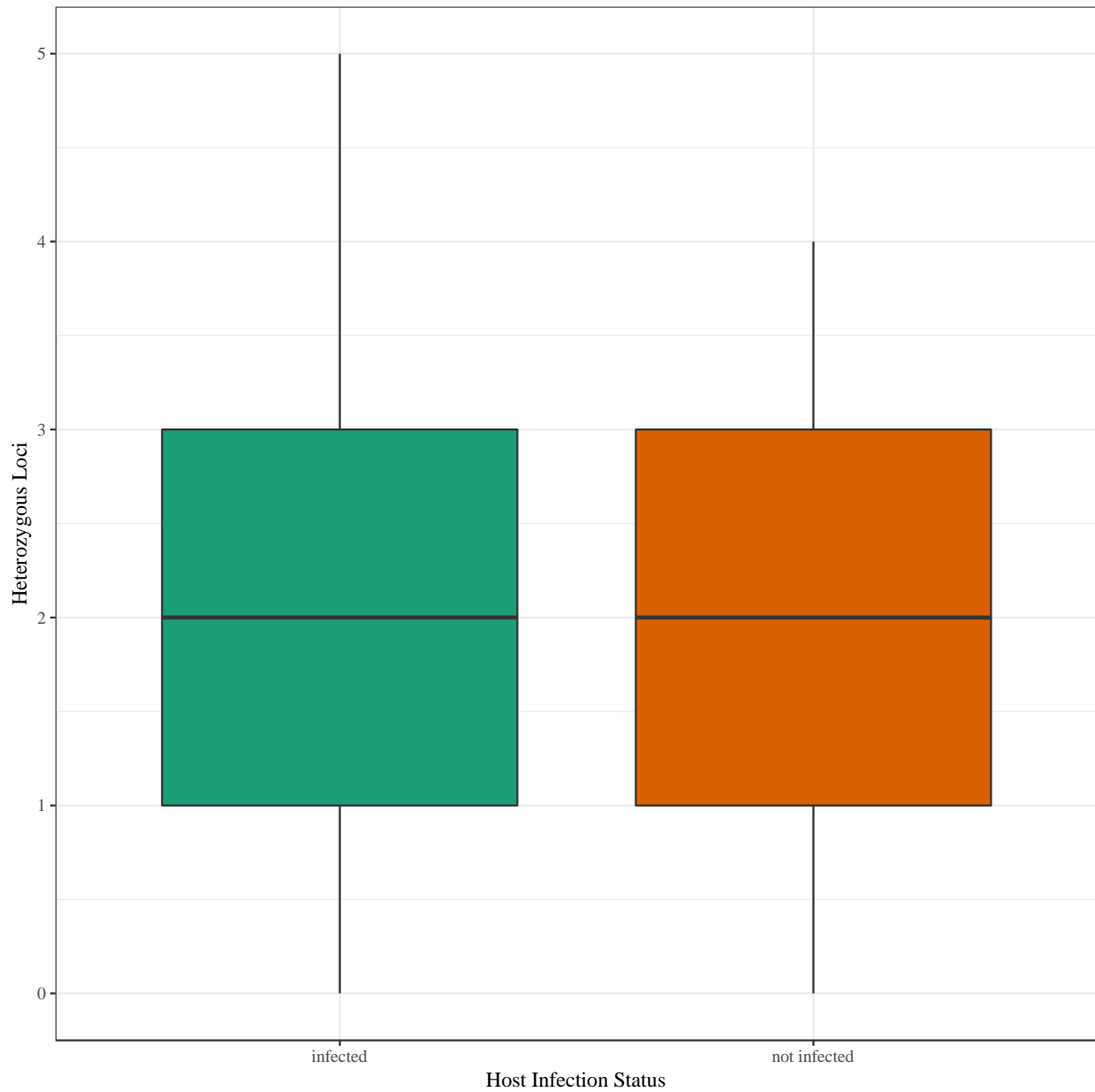


Figure 3. Average heterozygous loci of infected and not infected hybrid catostomids from the Santa Clara Watershed.

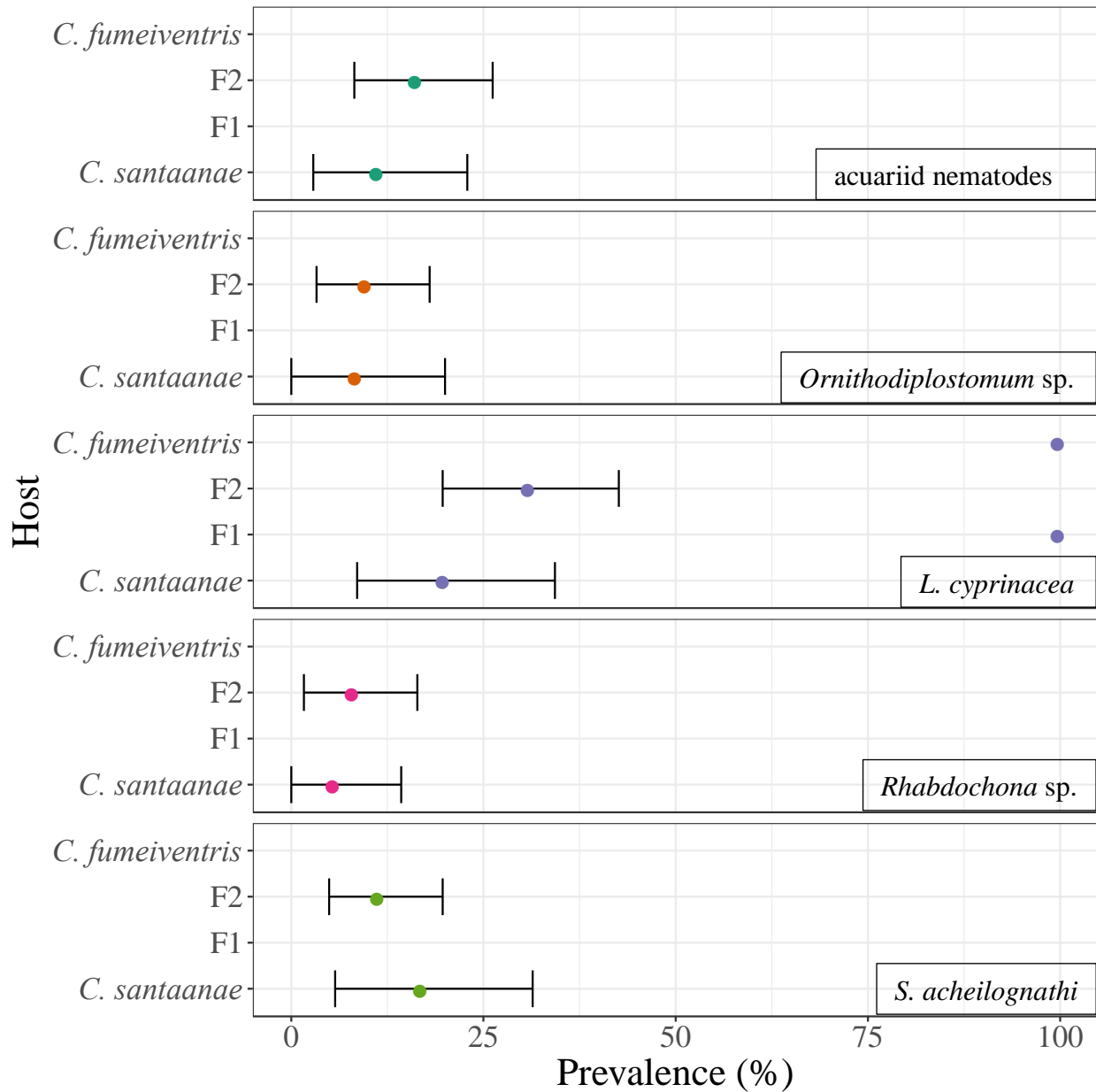


Figure 4. Prevalence of encysted acuariid nematodes, *Ornithodiplostomum* sp., *L. cyprinacea*, *Rhabdochona* sp., *S. acheilognathi* from catostomid hosts in the Santa Clara Watershed. There is no significant difference in prevalence between hosts. Error bars represent 95% bootstrapped confidence intervals.

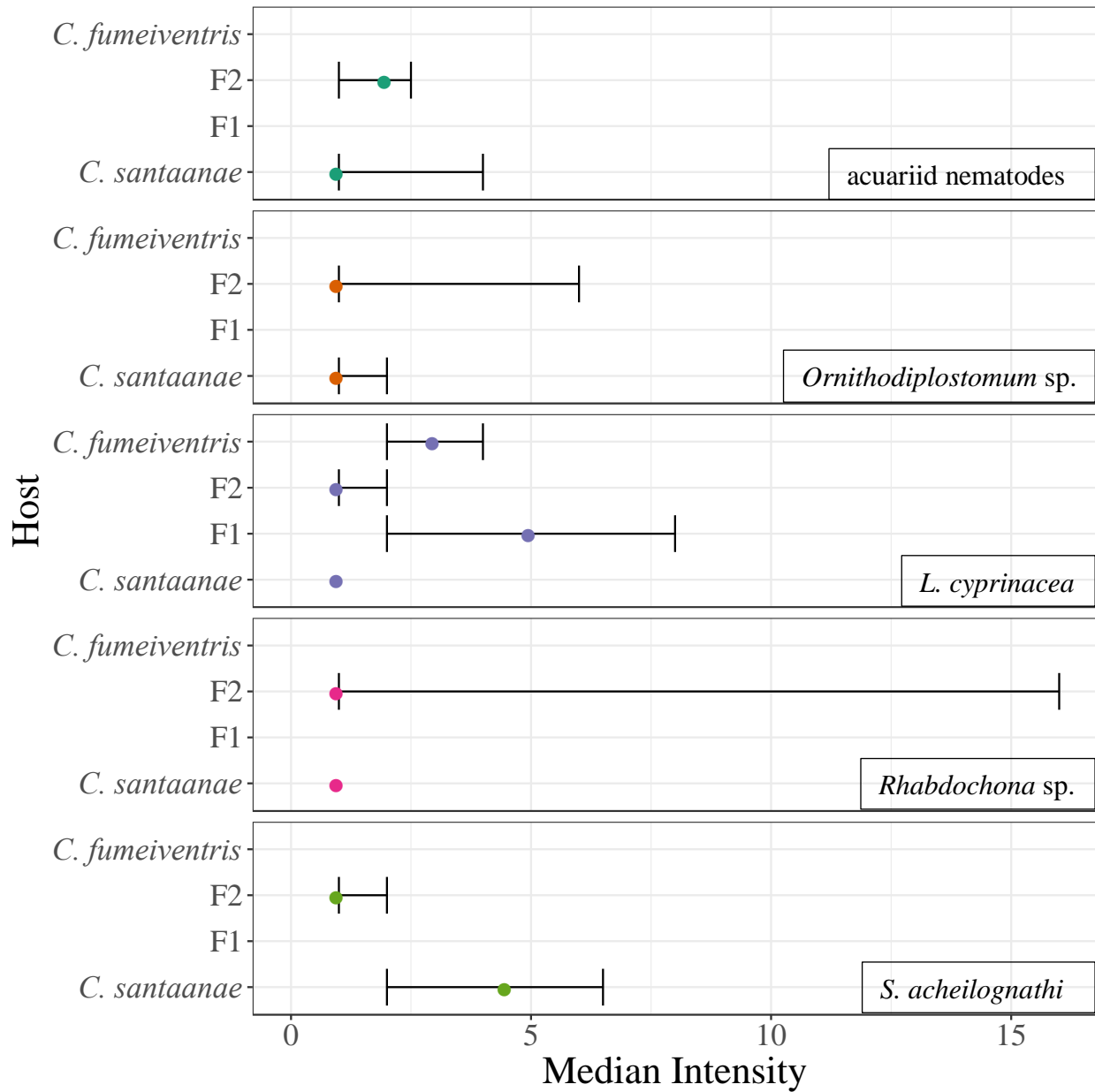


Figure 5. Median intensity of encysted acuariid nematodes, *Ornithodiplostomum* sp., *L. cyprinacea*, *Rhabdochona* sp., *S. acheilognathi* from catostomid hosts in the Santa Clara Watershed. There is no significant difference in median intensity between hosts. Error bars represent 95% bootstrapped confidence intervals.

## LITERATURE CITED

Amin, O.M. 1969. Helminth fauna of suckers (Catostomidae) of the Gila River System, Arizona.

II. Five parasites from *Catostomus* spp. American Midland Naturalist 82, 429 - 433.

<https://doi.org/10.2307/2423788>

Anderson, R.C. 1988. Nematode transmission patterns. Journal of Parasitology 74, 30 - 45.

<https://doi.org/10.2307/3282477>

Archdeacon, T.P., Iles, A., Kline, J.S., and S.A. Bonar. 2010. Asian fish tapeworm

*Bothriocephalus acheilognathi* in the desert southwestern United States. Journal of

Aquatic Animal Health 22, 274 - 279. <https://doi.org/10.1577/H09-009.1>

Bangs, M.R., Douglas, M.R., Mussmann, S.M., and M.E. Douglas. 2018. Unraveling historical introgression and resolving phylogenetic discord within *Catostomus* (Osteichthys:

Catostomidae). BMC Evolutionary Biology 18, 1–16. <https://doi.org/10.1186/s12862-018-1197-y>

Barber, I., Hoare, D., and J. Krause. 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries 10, 131–165.

Begnudelli, L., and B.F. Sanders. 2007. Simulation of the St. Francis Dam-break flood. Journal of Engineering Mechanics 133, 1200–1212. [https://doi.org/10.1061/\(ASCE\)0733-](https://doi.org/10.1061/(ASCE)0733-9399(2007)133:11(1200))

[9399\(2007\)133:11\(1200\)](https://doi.org/10.1061/(ASCE)0733-9399(2007)133:11(1200))

Bell, M.A. 1978. Fishes of the Santa Clara River System, Southern California. Natural History Museum of Los Angeles County, Los Angeles, California. Contributions in Science 295, 1 - 20.

Beller, E., Grossinger, R., Salomon, M., Dark, S., Stein, E., Orr, B., Downs, P., Longcore, T., Coffman, G., Whipple, A., Askevold, R., Stanford, B., and J. Beagle. 2011. Historical

- ecology of the lower Santa Clara River, Ventura River, and Oxnard Plain: an analysis of terrestrial, riverine, and coastal habitats (State Coastal Conservancy No. 641), Historical Ecology Program. San Francisco Estuary Institute, Oakland, CA.
- Bethel, W.M., and J.C. Holmes. 1973. Altered evasive behavior and responses to light in amphipods harboring Acanthocephalan cystacanths. *Journal of Parasitology* 59, 945–956.  
<https://doi.org/10.2307/3278623>
- Bray, A., Ismay, C., Baumer, B., and M. Cetinkaya-Rundel. 2019. infer: Tidy Statistical Inference. R package version 0.4.0.1. <https://CRAN.R-project.org/package=infer>
- Brooks, D.R., and E.P. Hoberg. 2000. Triage for the biosphere: The need and rationale for taxonomic inventories and phylogenetic studies of parasites. *Comparative Parasitology* 67, 1–25.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83, 575–583.  
<https://doi.org/10.2307/3284227>
- Buth, D.G., and C.B. Crabtree. 1982. Genetic variability and population structure of *Catostomus santaanae* in the Santa Clara Drainage. *Copeia* 1982, 439 - 444.  
<https://doi.org/10.2307/1444626>
- Buth, D.G., Sim, J., and C.C. Swift. 2008. Genetic confirmation of hybridization between *C. fumeiventris* and *C. santaanae* (Cypriniformes: Catostomidae) in the Santa Clara Drainage, in: Abstract Presented at the Southern California Academy of Sciences Annual Meeting.
- Chabot, C., Buth, D., Swift, C.C., Sim, J., Dowling, T., and L. Allen. 2009. Introgression of mitochondrial DNA between *Catostomus fumeiventris* and *Catostomus santaanae*

- (Cypriniformes: Catostomidae) in the Santa Clara Drainage, in: Abstract Presented at the Southern California Academy of Sciences Annual Meeting.
- Choudhury, A., Hoffnagle, T.L., and R.A. Cole. 2004. Parasites of native and nonnative fishes of the Little Colorado River, Grand Canyon, Arizona. *Journal of Parasitology* 90, 1042–1053. <https://doi.org/10.1645/GE-3244>
- Commonwealth Institute of Helminthology, 1974. CIH Keys to the Nematode Parasites of Vertebrates, 1st ed. Commonwealth Agricultural Bureaux, Farnham Royal, UK
- Crowley, P.H. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. *Annual Review of Ecology and Systematics* 23, 405–447.
- Dailey, M.D. 1996. Meyer, Olsen and Schmidt's Essentials of Parasitology. WC Brown Publishers, Dubuque, Iowa.
- Gotelli, N.J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81, 2606–2621.
- Grantham, T.E., Fesenmyer, K.A., Peek, R., Holmes, E., Quiñones, R.M., Bell, A., Santos, N., Howard, J.K., Viers, J.H., and P.B. Moyle. 2017. Missing the boat on freshwater fish conservation in California: California fish conservation planning. *Conservation Letters* 10, 77–85. <https://doi.org/10.1111/conl.12249>
- Greenfield, D., Ross, S., and G. Deckert. 1970. Some aspects of the life history of the Santa Ana sucker, *Catostomus (Pantosteus) santaanae* (Snyder). *California Fish and Game* 53, 166–179.
- Grolemund, G., and H. Wickham. 2011. Dates and times made easy with lubridate. *Journal of Statistical Software* 40, 1–25.



- Hansen, S.P., Choudhury, A., Heisey, D.M., Ahumada, J.A., Hoffnagle, T.L., and R.A. Cole. 2006. Experimental infection of the endangered bonytail chub (*Gila elegans*) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition. Canadian Journal of Zoology 84, 1383–1394. <https://doi.org/10.1139/z06-126>
- Henriksen, E.H., Frainer, A., Knudsen, R., Kristoffersen, R., Kuris, A.M., Lafferty, K.D., and P. Amundsen. 2019. Fish culling reduces tapeworm burden in Arctic charr by increasing parasite mortality rather than by reducing density-dependent transmission. Journal of Applied Ecology, 00, 1 - 10. <https://doi.org/10.1111/1365-2664.13369>
- Hoffman, G.L. 1958. Studies on the life-cycle of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeoidea) and the “self cure” of infected fish. Journal of Parasitology 44, 416–421. <https://doi.org/10.2307/3274326>
- Hoffman, G.L. 1999. Parasites of North American Freshwater Fishes. Cornell University Press, Ithica New York.
- Hossain, M.M., Ferdoushi, J., and A. Haque. 2018. Biology of anchor worms (*Lernaea cyprinacea*). Journal of Entomology and Zoology Studies 6, 910–917.
- Howard, J.K., Klausmeyer, K.R., Fesenmyer, K.A., Furnish, J., Gardali, T., Grantham, T., Katz, J.V.E., Kupferberg, S., McIntyre, P., Moyle, P.B., Ode, P.R., Peek, R., Quiñones, R.M., Rehn, A.C., Santos, N., Schoenig, S., Serpa, L., Shedd, J.D., Slusark, J., Viers, J.H., Wright, A., and S.A. Morrison. 2015. Patterns of freshwater species richness, endemism, and vulnerability in California. PLoS ONE 10, 1- 16. e0130710. <https://doi.org/10.1371/journal.pone.0130710>
- Hubbs, C.L. 1955. Hybridization between fish species in nature. Taylor and Francis, Ltd. for the Society of Systematic Biologists 4, 1–20.

Hudson, P.J., Dobson, A.P., and K.D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21, 381–385.

<https://doi.org/10.1016/j.tree.2006.04.007>

Jenkins, J.A., Goodbred, S.L., Olivier, H.M., Draugelis-Dale, R.O., and D.A. Alvarez. 2009. Effects of wastewater discharges on endocrine and reproductive function of western mosquitofish (*Gambusia* spp.) and implications for the threatened Santa Ana sucker (*Catostomus santaanae*) (U.S. Geological Survey Open File Report No. 2009–1097).

U.S. Geological Survey, Reston, Virginia. <https://doi.org/10.3133/ofr20091097>

Justine, J.-L., Briand, M.J., and R.A. Bray. 2012. A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. *Parasitology Research* 111, 341–351. <https://doi.org/10.1007/s00436-012-2845-6>

Kabata, Z. 1979. *Parasitic Copepoda of British Fishes*. The Ray Society, London, England.

Kock, R.A., Woodford, M.H., and P.B. Rossiter. 2010. Disease risks associated with the translocation of wildlife. *Revue Scientifique et Technique de l'OIE* 29, 329–350.

<https://doi.org/10.20506/rst.29.2.1980>

Kuchta, R., Choudhury, A., and T. Scholz. 2018. Asian fish tapeworm: The most successful invasive parasite in freshwaters. *Trends in Parasitology* 34, 511–523.

<https://doi.org/10.1016/j.pt.2018.03.001>

Kuperman, B.I., Matey, V.E., Warburton, M.L., and R.N. Fisher. 2002. Introduced parasites of freshwater fish in southern California, U.S.A. The Tenth International Congress of Parasitology - ICOPA X: Symposia, Workshops and Contributed Papers. August 4-9, 407–411.

- Kupferberg, S.J., Catenazzi, A., Lunde, K., Lind, A.J., and W.J. Palen. 2009. Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in Northern California. *Copeia* 2009, 529–537. <https://doi.org/10.1643/CH-08-011>
- Mackiewicz, J.S. 1972. Caryophyllidea (Cestoidea): A review. *Experimental Parasitology* 31, 417–512. [https://doi.org/10.1016/0014-4894\(72\)90103-8](https://doi.org/10.1016/0014-4894(72)90103-8)
- Marchetti, M.P., Moyle, P.B., and R. Levine. 2004. Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. *Freshwater Biology* 49, 646–661. <https://doi.org/10.1111/j.1365-2427.2004.01202.x>
- Matisz, C.E., and C.P. Goater. 2010. Migration, site selection, and development of *Ornithodiplostomum* sp. metacercariae (Digenea: Strigeoidea) in fathead minnows (*Pimephales promelas*). *International Journal for Parasitology* 40, 1489–1496. <https://doi.org/10.1016/j.ijpara.2010.04.018>
- Medeiros, E.S.F., and L. Maltchik. 1999. The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. *Journal of Arid Environments* 43, 351–356. <https://doi.org/10.1006/jare.1999.0545>
- Miller, R. 1973. Two new fishes, *Gila bicolor snyderi* and *Catostomus fumeiventris*, from the Owens River Basin, California. *Occasional Papers of the Museum of Zoology, University of Michigan* 667, 1–22.
- Moravec, F. 2010. Some aspects of the taxonomy, biology, possible evolution and biogeography of nematodes of the spirurine genus *Rhabdochona* Railliet, 1916 (Rhabdochonidae, Thelazioidea). *Acta Parasitologica* 55, 144–160. <https://doi.org/10.2478/s11686-010-0017-3>

- Moravec, F., D.G. Huffman. 1988. Observations on the genus *Rhabdochona* Railliet, 1926 (Nematoda: Rhabdochonidae) from fishes of central Texas, with descriptions of two new species. *Folia Parasitologica* 35, 341–351.
- Moyle, P.B. 1976. Fish introductions in California: History and impact on native fishes. *Biological Conservation* 9, 101–118. [https://doi.org/10.1016/0006-3207\(76\)90043-4](https://doi.org/10.1016/0006-3207(76)90043-4)
- Moyle, P.B. 2002. *Inland Fishes of California: Revised and Expanded*. University of California Press, Berkley and Los Angeles, California.
- Müller, K. 2018. hms: Pretty Time of Day. R package version 0.5.0. <https://CRAN.R-project.org/package=hms>
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Radabaugh, D.C. 1980. Changes in minnow, Rafinesque, schooling behaviour associated with infections of brainencysted larvae of the fluke, *Ornithodiplostomum ptychocheilus*. *Journal of Fish Biology* 16, 621–628. <https://doi.org/10.1111/j.1095-8649.1980.tb03741.x>
- Moyle, P.B. 1976. Fish introductions in California: History and impact on native fishes. *Biological Conservation* 9, 101–118. [https://doi.org/10.1016/0006-3207\(76\)90043-4](https://doi.org/10.1016/0006-3207(76)90043-4)
- Reiczigel, J., Marozzi, M., Fábíán, I., and L. Rózsa. 2019. Biostatistics for parasitologists – A primer to quantitative parasitology. *Trends in Parasitology* 35, 277–281. <https://doi.org/10.1016/j.pt.2019.01.003>
- Richmond, J.Q., Backlin, A.R., Galst-Cavalcante, C., O’Brien, J.W., and R.N. Fisher. 2018. Loss of dendritic connectivity in southern California’s urban riverscape facilitates decline of

- an endemic freshwater fish. *Molecular Ecology* 27, 369–386.  
<https://doi.org/10.1111/mec.14445>
- Robinson, A.T., Hines, P.P., Sorensen, J.A., and S.D. Bryan. 1998. Parasites and fish health in a desert stream, and management implications for two endangered fishes. *North American Journal of Fisheries Management* 18, 599–608. [https://doi.org/10.1577/1548-8675\(1998\)018<0599:PAFHIA>2.0.CO;2](https://doi.org/10.1577/1548-8675(1998)018<0599:PAFHIA>2.0.CO;2)
- Saiki, M.K., Martin, B.A., Knowles, G., and P. Tennant. 2007. Life history and ecological characteristics of the Santa Ana sucker, *Catostomus santaanae*. *California Fish and Game* 93, 87–101.
- Scholz, T., and A. Choudhury. 2014. Parasites of freshwater fishes in North America: Why so neglected? *Journal of Parasitology* 100, 26–45.
- Shields, R.J., and W.M. Tidd. 1974. Site selection on hosts by copepodids of *Lernaea cyprinacea* L. (Copepoda). *Crustaceana* 27, 225–230.
- Smith, G.R. 1992. Introgression in fishes: Significance for paleontology, cladistics, and evolutionary rates. *Systematic Biology* 41, 41–57. <https://doi.org/10.2307/2992505>
- Smith, G.R. 1966. Distribution and evolution of the North American catostomid fishes of the subgenus *Pantosteus*, Genus *Catostomus*. *Miscellaneous Publications Museum of Zoology University of Michigan* 129, 1–140.
- Thomas, F., and R. Poulin. 1997. Using randomization techniques to analyze fluctuating asymmetry data. *Animal Behaviour* 54, 1027–1029.  
<https://doi.org/10.1006/anbe.1997.0523>
- Thompson, A.R., Baskin, J.N., Swift, C.C., Haglund, T.R., and R.J. Nagel. 2010. Influence of habitat dynamics on the distribution and abundance of the federally threatened Santa Ana

- Sucker, *Catostomus santaanae*, in the Santa Ana River. *Environmental Biology of Fishes* 87, 321–332. <https://doi.org/10.1007/s10641-010-9604-2>
- Tufail, H., Qureshi, N.A., Khan, N., Iqbal, K.J., Khan, M.R., and A. Maqbool. 2017. Prevalence and effects of *Lernaea cyprinacea* (anchor worm) on the growth, skin histopathology and hematology of *Catla catla*. *Iranian Journal of Fisheries Sciences* 16, 341–351.
- Waicheim, M.A., Arbetman, M., Rauque, C., and G. Viozzi. 2019. The invasive parasitic copepod *Lernaea cyprinacea*: updated host-list and distribution, molecular identification and infection rates in Patagonia. *Aquatic Invasions* 14, 2, 350 - 364.  
<https://doi.org/10.3391/ai.2019.14.2.21>
- Warburton, M., Kuperman, B., and V. Matey. 2001. Parasite analysis of native and non-native fish in the Angeles National Forest (U.S. Forest Service, Angeles National Forest). Western Ecological Research Center U.S. Geological Survey, San Diego, California.  
<https://pubs.er.usgs.gov/publication/96889>
- Welicky, R.L., De Swardt, J., Gerber, R., Netherlands, E.C., and N.J. Smit. 2017. Drought-associated absence of alien invasive anchorworm, *Lernaea cyprinacea* (Copepoda: Lernaeidae), is related to changes in fish health. *International Journal for Parasitology: Parasites and Wildlife* 6, 430–438. <https://doi.org/10.1016/j.ijppaw.2017.01.004>
- Wickham, H. 2017. tidyverse: Easily Install and Load the “Tidyverse.” R package version 1.2.1.  
<https://CRAN.R-project.org/package=tidyverse>
- Wier, W., Mayberry, L.F., Kinzer, H.G., and P.R. Turner. 1983. Parasites of fishes in the Gila River Drainage in southwestern New Mexico. *Journal of Wildlife Diseases* 19, 59–60.  
<https://doi.org/10.7589/0090-3558-19.1.59>

## **Chapter 2 - Macroparasites of *Gila orcutti* and *Pimephales promelas* (Actinopterygii: Cyprinidae) in the Santa Clara River, Ventura County, California.**

### **INTRODUCTION**

Parasites have been historically excluded from ecological and evolutionary research surrounding freshwater fishes of North America (Scholz and Choudhury, 2014). Parasitologists conducting their research in Mexico and Canada have concentrated their efforts on parasites of freshwater fishes, however there have only been a handful of parasite studies focused on the parasites of freshwater fishes in the southwestern United States (Robinson et al., 1998; Choudhury et al., 2004; Archdeacon et al., 2010). The western United States, with the exception of the Pacific Northwest, is largely dominated by an arid climate with river systems that have highly variable flow regimes with a large number of endemic aquatic organisms (Smith, 1978; Moyle, 1995; Beller et al., 2011). The interaction of the region's climate, habitat alterations, and species introductions has led to extinction and population decreases in endemic fishes (Grantham et al., 2017).

Fourteen species of chub in the genus *Gila* range from the western United States to northern Mexico with several of these species being classified as threatened, endangered, or extinct (Page and Burr, 2011). The arroyo chub, *Gila orcutti*, is the only extant species found in coastal California and is listed as a species of special concern in its native range in southern California (Moyle, 2002; Page and Burr, 2011). The populations of *G. orcutti* within its native range are in decline while introduced populations in the Santa Clara River are sustained (Moyle, 2002). There are other introduced cyprinids in the Santa Clara River, that were imported from

the eastern United States and Asia including: *Pimephales promelas* (fathead minnow), *Cyprinus carpio* (common carp), *Notemigonus crysoleucas* (golden shiner) (Moyle, 2002).

Although both *G. orcutti* and *P. promelas* are introduced to the Santa Clara River, the population of *G. orcutti* was likely translocated by recreational anglers from the adjacent drainages whereas the population of *P. promelas* was introduced from the eastern United States (Moyle, 2002). A parasite survey of fishes in this region has been limited to the detection and distribution of three nonnative pathogenic parasites: *Ichthyophthirius multifiliis* (white spot disease), *Schyzocotyle acheilognathi* (Asian fish tapeworm), and *Lernaea cyprinacea* (anchor worm) (Warburton et al., 2001; Kuperman et al., 2002). Describing the host associations of these three pathogenic parasites is important for management and recovery efforts; however, native parasites have been neglected from these studies and can be useful bioindicators of community structure, population structure, trophic interactions, and biogeography (Brooks and Hoberg, 2000). This study identifies the macroparasite fauna that is associated with *G. orcutti* and *P. promelas* in the Santa Clara River in an effort to understand potential threats posed by nonnative pathogenic parasites as well as describe the native parasite fauna in this watershed. Additionally, this study will compare the prevalence and median intensity of the shared parasites between *G. orcutti* and *P. promelas*.

## **MATERIALS AND METHODS**

The study area included two sampling sites, the mainstem of the Santa Clara River and Sespe Creek, in Ventura County, California. The sampling locality in the mainstem of the river was characterized by a dense riparian buffer that consisted of large willows and Spanish cane that created a canopy over most of the river. The banks were dominated by emergent vegetation



and deep undercuts in slower portions of the river. Sespe Creek was characterized by an open canopy with a narrow corridor of willows bordering the creek. The banks were steep with sparse emergent vegetation. The sampling efforts in Sespe Creek took place from June to September in 2017 and from May to September in 2018. The mainstem of the Santa Clara River was only able to be sampled during August and September of 2018.

Fishes were caught using a 5 m two-person seine net with a nylon delta weave 5 mm mesh size. In accordance with the California Department of Fish and Wildlife a specialized seine protocol was developed in order to minimize disturbance to potential southern California steelhead habitat. Sampling efforts were limited to fifty seine hauls in each 50-meter reach. Additionally, sampling efforts were restricted to one effort per month during the sampling period. Lethal take was highly regulated by the California Department of Fish and Wildlife with the maximum number of *G. orcutti* being limited to 35 individuals per sampling effort. Fishes were identified, counted, and placed in 19 L buckets with aerators before processing. Standard length was measured then the fishes were necropsied using dissecting microscopes.

External surfaces, and internal organs including the stomach, intestine, and gallbladder were examined for parasites. Parasites were fixed and preserved using methods described in Dailey (1996) and Justine et al. (2012). Platyhelminths, acanthocephalans, and nematodes were preserved for both morphological and molecular analysis. Copepods were collected and placed directly into either 70% or 95% ethanol. Parasites were identified with Commonwealth Institute of Helminthology (1974), Kabata (1979), and Hoffman (1999).

Parasite prevalence, mean intensity, and median intensity were calculated for each host (Bush et al., 1997). Parasite distributions were aggregated, thus rendering mean intensity an inaccurate descriptor of parasite intensity in the host population (Reiczigel et al., 2019).

Permutation tests were used to analyze differences in prevalence, and median intensity between hosts (Crowley, 1992; Thomas and Poulin, 1997; Gotelli, 2000). Observed values were randomly shuffled without replacement keeping sample size constant for 10,000 iterations. Difference values of each iteration were compiled to create a distribution of potential differences. Observed differences in prevalence and median intensity were then compared to their respective probability distributions from the permutation to obtain a probability value. Bootstrapped ninety-five percent confidence intervals were calculated with 10,000 iterations for prevalence and median intensity values. Median intensity confidence intervals were displayed when parasite intensity was variable. Data analysis was performed with the “tidyverse” and “infer” packages in R (Wickham, 2017; Bray et al., 2018).

## RESULTS

During this study a total of 214 *G. orcutti* and 18 *P. promelas* were collected from the lower Santa Clara Watershed. The internal parasite fauna consisted of six taxa including: one cestode (*Schyzocotyle acheilognathi*), one digenean (diplostomid metacercariae), three nematodes (*Rhabdochona* sp., *Contracaecum* sp., and larval acuariid cysts), and one acanthocephalan (unidentified cystacanth). The external parasite fauna consisted of two taxa including: one monogenean (*Gyrodactylus* sp.) and one copepod (*Lernaea cyprinacea*). All eight parasite taxa were found associated with *G. orcutti* while only the adult tapeworm *S. acheilognathi*, diplostomid metacercariae, larval acuariid cysts, and *L. cyprinacea* were associated with *P. promelas* (Table 1). Intensity of infection was not consistently related to the standard length of the hosts ( $X^2 = 2.9736$ ,  $df = 1$ ,  $P = 0.0562$ ). The prevalence of *S. acheilognathi* was 13.55% and 22.22% in *G. orcutti* and *P. promelas*, respectively, with no significant

difference in prevalence between the two hosts ( $p = 0.469$ ) (Table 1). Additionally, there was no significant difference in the median intensity of *S. acheilognathi* between hosts ( $p = 1.00$ ) (Table 1). The prevalence of diplostomid metacercariae was significantly different between hosts being 1.87% in *G. orcutti* and 94.44% in *P. promelas* ( $p < 0.0001$ ) (Table 1). The median intensity of the metacercariae from *G. orcutti* was 3 while it was 106 in *P. promelas* ( $p < 0.0001$ ) (Table 1). Encysted acuariid nematodes had similar prevalence and median intensity in both hosts ( $p = 1.00$ ). The prevalence of adult *L. cyprinacea* was significantly higher in *G. orcutti* than in *P. promelas* ( $p = 0.042$ ) (Table 1). In contrast, the prevalence of the copepodid stage of *L. cyprinacea* was significantly lower in *G. orcutti* than in *P. promelas* ( $p < 0.0001$ ) (Table 1). However, median intensity of adult *L. cyprinacea* was not significantly different between hosts ( $p = 1.00$ ) (Table 1). While the median intensity of *Lernaea cyprinacea* copepodids was higher in *G. orcutti* than in *P. promelas* it was not statistically different ( $p = 0.450$ ) (Table 1).

## DISCUSSION

This study reports seven new parasite associations for *G. orcutti* and one new parasite association for *P. promelas*. Although *L. cyprinacea* and *S. acheilognathi* had been reported from *G. orcutti* and *P. promelas*, this study reports a new record of *L. cyprinacea* infecting *P. promelas* in the Santa Clara River in addition to new records of associations of *S. acheilognathi* with *G. orcutti* and *P. promelas* in the Santa Clara River (Warburton et al., 2001; Kuperman et al., 2002). The observed prevalence and intensity of *S. acheilognathi* in both hosts were similar to other studies in the southwestern United states (Robinson et al., 1998; Choudhury et al., 2004; Archdeacon et al. 2010). Cyprinids are one of the most commonly infected final hosts for this species of tapeworm; for this reason, the recovery and conservation of cyprinids of the south

western United States are at risk due to infection of *S. acheilognathi* (Clarkson et al., 1997; Hansen et al., 2006; Kuchta et al., 2018).

This study is one of the first to report the infection parameters of both adult and copepodid stages of *L. cyprinacea* in the Santa Clara River. While the prevalence of adult *L. cyprinacea* was higher in *G. orcutti* than in *P. promelas* the median intensity was not significantly different. This disparity could be due to the low sample size and relative abundance of *P. promelas* in the Santa Clara River. While the intensity of adult *L. cyprinacea* was relatively low, the location of that copepod imbedded on the host could negatively affect the swimming performance (Medeiros and Maltchik, 1999; Welicky et al., 2017). The copepodid stages of *L. cyprinacea* were more prevalent on *P. promelas* than on *G. orcutti*, however the copepodid stages are still highly mobile and can swim from host to host which may explain this observed inconsistency between prevalence and median intensity. The effects of the copepodid stages of *L. cyprinacea* are not well described, however some research correlated intensity of copepodid infection with damage to fish gill filaments and fish die-offs (Shields and Tidd, 1974; Hossain et al., 2018).

The digeneans, acuariid nematodes, species of *Contracaecum* sp., and the unidentified cystacanth that were collected during this study are known to complete their complex life cycles in picivorous birds (Anderson, 1988; Hoffman, 1958, 1999). The most abundant taxa of parasite were diplostomid metacercariae that were collected from both hosts, but had a significantly higher prevalence and median intensity in *P. promelas*. The metacercariae are difficult to identify at this stage, however during dissection some metacercariae excysted and were subsequently identified as two species: one in the genus *Ornithodiplostomum* and the other in the genus *Posthodiplostomum*. Metacercariae from both genera had been reported from *P. promelas*

as well as other cyprinids in the southwestern United States (Radabaugh, 1980; Robinson et al., 1998; Choudhury et al., 2004; Matisz and Goater, 2010). Although these specimens had not been identified to the species level, some species in this group of digeneans have been known to encyst on the brain of fish hosts and manipulate host behavior (Hoffman, 1958; Matisz and Goater, 2010; Radabaugh, 1980). Both larval nematodes (*Contracaecum* sp. and encysted acuariids) are difficult to identify to the species level at this developmental stage. The acuariid cysts were collected from the intestinal viscera, and the larval *Contracaecum* sp. were collected from the lumen of the intestine are both common parasites of aquatic birds (Anderson, 1988; Commonwealth Institute of Helminthology, 1974). These nematodes along with the digeneans, and acanthocephalans collected during this study show that freshwater fishes in southern California are integral linkages in the life cycles of these parasites and shed light on the trophic interactions between piscivorous birds and the fishes in the Santa Clara River.

One species of monogenean (*Gyrodactylus* sp.) was collected during this study from the gill filaments of *G. orcutti*, which represents the first known record of a monogenean collected from this host. Monogeneans in the genus *Gyrodactylus* can be highly host specific and are often used as a model organism for coevolutionary studies (Gilmore et al., 2012). These specimens may represent a new species, but additional research is needed to confirm the identity. Some species of *Gyrodactylus* are known to be pest in aquaculture and can lead to fish kills in commercial settings (Harris et al., 2000).

One species of nematode in the genus *Rhabdochona* was the only adult collected during this study. Species in this genus are widespread in freshwater fishes of the world and are especially interesting due to their relatively high host specificity (Mejía-Madrid et al. 2007; Moravec, 2010). This group of nematodes has been described to have a life cycle that is

comprised of an aquatic insect intermediate host with the final host being a fish (Anderson, 1988). Larvae, adult males and gravid females were collected from *G. orcutti* during this study, which suggests that this nematode can complete its life cycle in this host. The morphological features of these specimens of *Rhabdochona* are similar to other species that are closely associated with cyprinids in North America, but additional morphological and molecular research is needed to confirm the identity of these specimens (Moravec, 2010; Moravec and Huffman, 1988).

This study provides a comprehensive survey of the macroparasites of *G. orcutti* and *P. promelas* in the Santa Clara River. The information reported here is useful for parasitologists and provides information about the infection parameters and distribution of two pathogenic parasites: *S. acheilognathi* and *L. cyprinacea*. Additionally, research focused on the parasites of freshwater fishes in southern California is important due to the high levels of endemism in the region (Scholz and Choudhury, 2014; Howard et al., 2015). Many organisms that inhabit the highly modified urban area of the southwestern United States are at risk and continuing research on the associated parasite fauna may lead to breakthroughs in recovery efforts.

Table 1. Prevalence % (P ), mean intensity (MI), median intensity (Med I), minimum - maximum counts (Min - Max), of parasites collected from cypriniform fishes in the Santa Clara River.

Host	<i>G. orcutti</i> SL: 30 - 85 (mm)				<i>P. promelas</i> SL: 35 - 54 (mm)			
Parasite	P	MI	Med I	Minimum- Maximum	P	MI	Med I	Minimum - Maximum
Monogenea								
<i>Gyrodactylus</i> sp.	2.80	4	1.5	0 - 16				
Cestoda								
<i>Schyzocotyle acheilognathi</i>	13.55	3.76	2	0 - 22	22.22	3	1.5	0 - 8
Digenea								
Diplostomid metacercariae	1.87	4.75	3	0 - 12	94.44	136	106	0 - 480
Acanthocephala								
Unidentified cystacanth	1.87	1	1	0 - 1				
Nematoda								

<i>Rhabdochona</i> sp.	68.69	8.39	5	0 - 110				
Acuariid cysts	7.94	1.41	1	0 - 4	5.56	1	1	0 - 1
<i>Contracaecum</i> sp.	6.07	1.38	1	0 - 4				
Copepoda								
<i>Lernaea cyprinacea</i> (adult)	28.97	1.73	1	0 - 5	5.56	1	1	0 - 1
<i>Lernaea cyprinacea</i> (copepodid)	1.40	6.67	4	0 - 14	33.33	1.5	1	0 - 4



## LITERATURE CITED

- Anderson, R.C. 1988. Nematode transmission patterns. *Journal of Parasitology* 74, 30–45.  
<https://doi.org/10.2307/3282477>
- Archdeacon, T.P., Iles, A., Kline, J.S., and S.A. Bonar. 2010. Asian fish tapeworm *Bothriocephalus acheilognathi* in the desert southwestern United States. *Journal of Aquatic Animal Health* 22, 274–279. <https://doi.org/10.1577/H09-009.1>
- Beller, E., Grossinger, R., Salomon, M., Dark, S., Stein, E., Orr, B., Downs, P., Longcore, T., Coffman, G., Whipple, A., Askevold, R., Stanford, B., and J. Beagle. 2011. Historical Ecology of the lower Santa Clara River, Ventura River, and Oxnard Plain: an analysis of terrestrial, riverine, and coastal habitats (State Coastal Conservancy No. 641), Historical Ecology Program. San Francisco Estuary Institute, Oakland, CA.
- Bray, A., Ismay, C., Baumer, B., and M. Cetinkaya-Rundel. 2019. infer: Tidy Statistical Inference. R package version 0.4.0.1. <https://CRAN.R-project.org/package=infer>
- Brooks, D.R., E.P. Hoberg. 2000. Triage for the biosphere: The need and rationale for taxonomic inventories and phylogenetic studies of parasites. *Comparative Parasitology* 67, 1–25.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology* 83, 575–583.  
<https://doi.org/10.2307/3284227>
- Choudhury, A., Hoffnagle, T.L., and R.A. Cole. 2004. Parasites of native and nonnative fishes of the Little Colorado River, Grand Canyon, Arizona. *Journal of Parasitology* 90, 1042–1053. <https://doi.org/10.1645/GE-3244>

- Clarkson, R.W., Robinson, A.T., and T.L. Hoffnagle. 1997. Asian tapeworm (*Bothriocephalus acheilognathi*) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Naturalist 57, 66 - 69. <https://www.jstor.org/stable/41712976>
- Commonwealth Institute of Helminthology. 1974. CIH Keys to the Nematode Parasites of Vertebrates, 1st ed. Commonwealth Agricultural Bureaux.
- Crowley, P.H. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. Annual Review of Ecology and Systematics 23, 405–447.
- Dailey, M.D. 1996. Meyer, Olsen and Schmidt's Essentials of Parasitology. WC Brown Publishers, Dubuque, Iowa.
- Gilmore, S.R., Cone, D.K., Lowe, G., King, S.K., Jones, S.R.M., and C.L. Abbott. 2012. Molecular phylogeny of *Gyrodactylus* (Monogenea) parasitizing fishes in fresh water, estuarine, and marine habitats in Canada. Canadian Journal of Zoology 90, 776–786. <https://doi.org/10.1139/z2012-040>
- Gotelli, N.J. 2000. Null model analysis of species co-occurrence patterns. Ecology 81, 2606–2621.
- Grantham, T.E., Fesenmyer, K.A., Peek, R., Holmes, E., Quiñones, R.M., Bell, A., Santos, N., Howard, J.K., Viers, J.H., and P.B. Moyle. 2017. Missing the boat on freshwater fish conservation in California: California fish conservation planning. Conservation Letters 10, 77–85. <https://doi.org/10.1111/conl.12249>
- Hansen, S.P., Choudhury, A., Heisey, D.M., Ahumada, J.A., Hoffnagle, T.L., and R.A. Cole. 2006. Experimental infection of the endangered bonytail chub (*Gila elegans*) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition. Canadian Journal of Zoology 84, 1383–1394. <https://doi.org/10.1139/z06-126>

- Harris, P.D., Soleng, A., and T.A. Bakke. 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* 120, 57–64. <https://doi.org/10.1017/S0031182099005223>
- Hoffman, G.L. 1958. Studies on the life-cycle of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeoidea) and the “self cure” of infected fish. *Journal of Parasitology* 44, 416–421. <https://doi.org/10.2307/3274326>
- Hoffman, G.L. 1999. *Parasites of North American Freshwater Fishes*. Cornell University Press, Ithaca, New York.
- Hossain, M.M., Ferdoushi, J., and A. Haque. 2018. Biology of anchor worms (*Lernaea cyprinacea*). *Journal of Entomology and Zoology Studies* 6, 910–917.
- Howard, J.K., Klausmeyer, K.R., Fesenmyer, K.A., Furnish, J., Gardali, T., Grantham, T., Katz, J.V.E., Kupferberg, S., McIntyre, P., Moyle, P.B., Ode, P.R., Peek, R., Quiñones, R.M., Rehn, A.C., Santos, N., Schoenig, S., Serpa, L., Shedd, J.D., Slusark, J., Viers, J.H., Wright, A., and S.A. Morrison. 2015. Patterns of freshwater species richness, endemism, and vulnerability in California. *PLoS ONE* 10, 1- 16. e0130710. <https://doi.org/10.1371/journal.pone.0130710>
- Justine, J.-L., Briand, M.J., and R.A. Bray. 2012. A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. *Parasitology Research* 111, 341–351. <https://doi.org/10.1007/s00436-012-2845-6>
- Kabata, Z. 1979. *Parasitic Copepoda of British Fishes*. The Ray Society, London, England.
- Kuchta, R., Choudhury, A., and T. Scholz. 2018. Asian fish tapeworm: The most successful invasive parasite in freshwaters. *Trends in Parasitology* 34, 511–523. <https://doi.org/10.1016/j.pt.2018.03.001>

- Kuperman, B.I., Matey, V.E., Warburton, M.L., and R.N. Fisher. 2002. Introduced parasites of freshwater fish in southern California, U.S.A. The Tenth International Congress of Parasitology 407–411.
- Matisz, C.E., and C.P. Goater. 2010. Migration, site selection, and development of *Ornithodiplostomum* sp. metacercariae (Digenea: Strigeoidea) in fathead minnows (*Pimephales promelas*). International Journal for Parasitology 40, 1489–1496.  
<https://doi.org/10.1016/j.ijpara.2010.04.018>
- Medeiros, E.S.F., and L. Maltchik. 1999. The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. Journal of Arid Environments 43, 351–356. <https://doi.org/10.1006/jare.1999.0545>
- Mejía-Madrid, H.H., Choudhury, A., and G.P.-P. de León. 2007. Phylogeny and biogeography of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) species from the Americas. Systematic Parasitology 67, 1–18. <https://doi.org/10.1007/s11230-006-9065-3>
- Moravec, F., and D.G. Huffman. 1988. Observations on the genus *Rhabdochona* Railliet, 1926 (Nematoda: Rhabdochonidae) from fishes of central Texas, with descriptions of two new species. Folia Parasitologica 35, 341–351.
- Moravec, F. 2010. Some aspects of the taxonomy, biology, possible evolution and biogeography of nematodes of the spirurine genus *Rhabdochona* Railliet, 1916 (Rhabdochonidae, Thelazioidea). Acta Parasitologica 55, 144–160. <https://doi.org/10.2478/s11686-010-0017-3>
- Moyle, P.B. 1995. Conservation of native freshwater fishes in the Mediterranean-type climate of California, USA: A review. Biological Conservation 72, 271–279.  
[https://doi.org/10.1016/0006-3207\(94\)00089-9](https://doi.org/10.1016/0006-3207(94)00089-9)

- Moyle, P.B. 2002. Inland Fishes of California: Revised and Expanded. University of California Press, Berkeley and Los Angeles, California.
- Page, L.M., and B.M. Burr. 2011. Peterson Field Guide to Freshwater Fishes of North America of Mexico. Houghton Mifflin Harcourt Publishing Company, New York, New York.
- Radabaugh, D.C. 1980. Changes in minnow, *Pimephales promelas* Rafinesque, schooling behaviour associated with infections of brain-encysted larvae of the fluke, *Ornithodiplostomum ptychocheilus*. Journal of Fish Biology 16, 621–628.  
<https://doi.org/10.1111/j.1095-8649.1980.tb03741.x>
- Reiczigel, J., Marozzi, M., Fábíán, I, and L. Rózsa. 2019. Biostatistics for parasitologists – A primer to quantitative parasitology. Trends in Parasitology 35, 277–281.  
<https://doi.org/10.1016/j.pt.2019.01.003>
- Robinson, A.T., Hines, P.P., Sorensen, J.A., and S.D. Bryan. 1998. Parasites and fish health in a desert stream, and management implications for two endangered fishes. North American Journal of Fisheries Management 18, 599–608. [https://doi.org/10.1577/1548-8675\(1998\)018<0599:PAFHIA>2.0.CO;2](https://doi.org/10.1577/1548-8675(1998)018<0599:PAFHIA>2.0.CO;2)
- Scholz, T., and A. Choudhury. 2014. Parasites of freshwater fishes in North America: Why so neglected? Journal of Parasitology 100, 26–45.
- Shields, R.J., and W.M. Tidd. 1974. Site selection on hosts by copepodids of *Lernaea cyprinacea* L. (Copepoda). Crustaceana 27, 225–230.
- Smith, G.R. 1978. Biogeography of intermountain fishes. Great Basin Naturalist Memoirs 2, 17 - 42. <https://www.jstor.org/stable/23376557>

- Thomas, F., and R. Poulin. 1997. Using randomization techniques to analyze fluctuating asymmetry data. *Animal Behaviour* 54, 1027–1029.  
<https://doi.org/10.1006/anbe.1997.0523>
- Warburton, M., Kuperman, B., and V. Matey. 2001. Parasite analysis of native and non-native fish in the Angeles National Forest (U.S. Forest Service, Angeles National Forest). Western Ecological Research Center U.S. Geological Survey, San Diego, California.  
<https://pubs.er.usgs.gov/publication/96889>
- Welicky, R.L., De Swardt, J., Gerber, R., Netherlands, E.C., and N.J. Smit. 2017. Drought-associated absence of alien invasive anchorworm, *Lernaea cyprinacea* (Copepoda: Lernaeidae), is related to changes in fish health. *International Journal for Parasitology: Parasites and Wildlife* 6, 430–438. <https://doi.org/10.1016/j.ijppaw.2017.01.004>
- Wickham, H. 2017. tidyverse: Easily Install and Load the “Tidyverse.” R package version 1.2.1.  
<https://CRAN.R-project.org/package=tidyverse>